Synthesis and Biological Activities of 10-Substituted Benzo[*b*][1,5]naphthyridines

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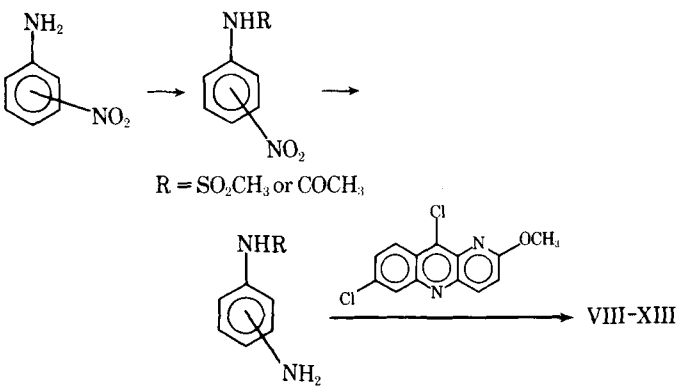
Abstract □ Eight 10-substituted benzo[*b*][1,5]naphthyridine derivatives containing *N*-(pyrrolidino)alkylamines, methanesulfonanilides, and aminoacetanilides were prepared, and their binding with DNA was studied by (*a*) T_m measurements and (*b*) the effect on DNA-dependent RNA polymerase *in vitro*. In addition, they were evaluated as antineoplastic agents in the P-388 test. None of the compounds exhibited anticancer activity.

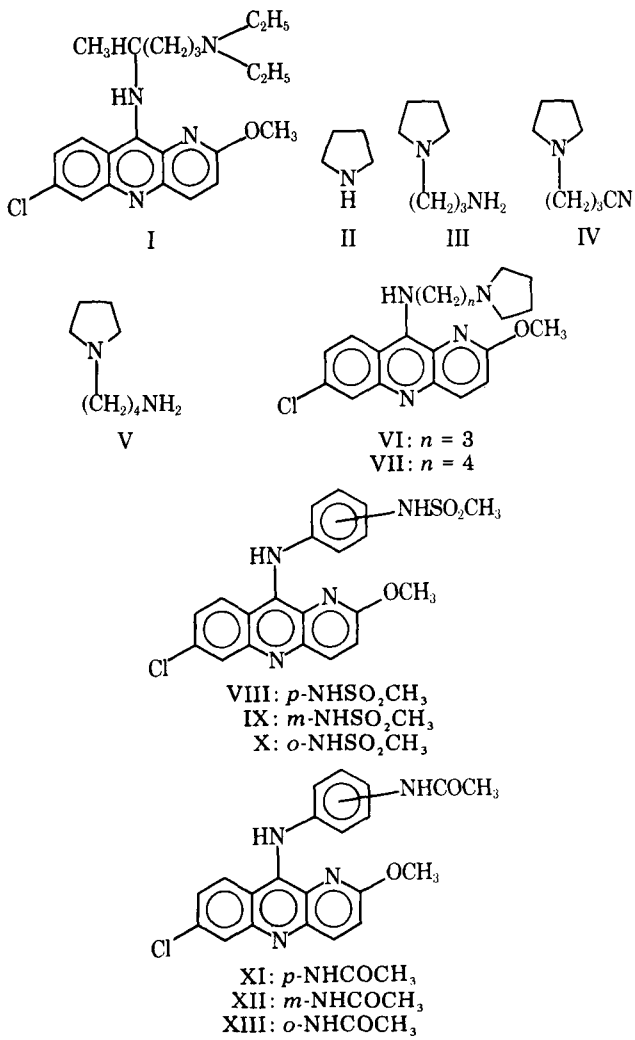
Keyphrases □ Benzo[*b*][1,5]naphthyridines, substituted—synthesized, binding with DNA studied, antitumor activity evaluated □ Binding—DNA to various substituted benzo[*b*][1,5]naphthyridines □ DNA—binding to various substituted benzo[*b*][1,5]naphthyridines □ Antitumor activity—various benzo[*b*][1,5]naphthyridines evaluated □ Structure–activity relationships—various substituted benzo[*b*][1,5]naphthyridines, binding with DNA studied, antitumor activity evaluated

The biological properties of acridine derivatives are well documented (1–5). These effects are believed to result from the intercalation of the acridine derivatives into DNA, inhibiting the ability of DNA to act as a template in DNA replication and RNA synthesis (6). Azacrin (I), structurally related to acridines and regarded as a combination of an 8-aminoquinoline and a 4-aminoquinoline, was synthesized (7) as an antimalarial. It was shown that I is an effective schizontocide in *Plasmodium falciparum* infection (8).

Although a large number of benzo[*b*][1,5]naphthyridine derivatives have been prepared (9, 10) and screened as antimalarial, antibacterial, and antineoplastic agents, the mechanism of action of these drugs remains poorly defined. Therefore, some 10-substituted benzo[*b*][1,5]naphthyridines (VI–XIII) were prepared and their ability to bind to DNA was studied by the evaluation of T_m (the temperature at which 50% hyperchromicity is attained owing to heat denaturation of native DNA) and their effect on *Escherichia coli* DNA-dependent RNA polymerase. In addition, these compounds were screened as potential antineoplastic agents.



Scheme I



RESULTS AND DISCUSSION

Chemistry—The intermediate *N*-(δ-aminopropyl)pyrrolidine (III) for the synthesis of VI was prepared from pyrrolidine by a cyanoethylation reaction with acrylonitrile, followed by reduction with lithium aluminum hydride. The intermediate V was prepared from II and 4-chlorobutyronitrile according to the method of Burckhalter *et al.* (11), followed by lithium aluminum hydride reduction (12) of the nitrile (IV).

The intermediates, aminomethanesulfonanilides for VIII–X and aminoacetanilides for XI–XIII, were prepared in excellent yields from the corresponding nitroanilines by standard chemical reactions. The desired target compounds, VIII–XIII, were then prepared (Scheme I) by reacting the appropriate amines with 7,10-dichloro-2-methoxypyrido[3,2-*b*]quinoline according to the method of Besly and Goldberg (7).

Biological Activity—The T_m studies were carried out according to

Table I—T_m Determination[a]

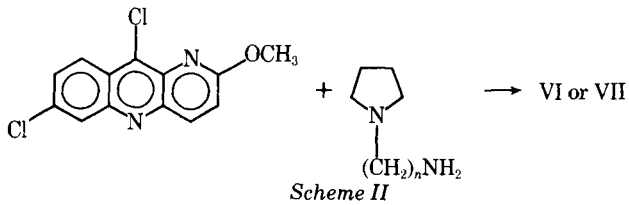
Compound	T_m	ΔT_m
DNA	68.5°	—
DNA + VI	83.5°	15.0°
DNA + VII	87.5°	19.0°
DNA + VIII	70.5°	2.0°
DNA + IX	70.0°	1.5°
DNA + X	70.5°	2.0°
DNA + XI	72.5°	4.0°
DNA + XII	68.5°	0
DNA + XIII	69.0°	0.5°

[a] The drugs were used at a concentration of 1×10^{-5} *M* in a solution containing 30 μg of calf thymus DNA/ml in 5 m*M* phosphate buffer at pH 7.4; the melting temperatures were determined at 260 nm by means of a Gilford 250 recording spectrophotometer with a thermoprogrammer 2527 programmed for a temperature rise of 1.0°/min.

Table II—Effect of 10-Substituted Benzo[*b*][1,5]naphthyridine on *E. coli* DNA-Primed RNA Polymerase

Drug Concentration, μg/ml	RNA Polymerase Activity[a], % Control							
	VI	VII	VIII	IX	X	XI	XII	XIII
400	0	0	111	116	145	153	178	186
200	1	0	134	145	113	190	153	182
100	10	1	135	119	153	143	112	173
50	23	19	111	128	140	138	139	163
25	42	30	126	147	150	124	123	126

[a] RNA polymerase activity was assayed as described under *Experimental*. All assays were run in triplicate. The results represent the average of two separate experiments that did not differ by more than 5%.



Scheme II

literature methods (13–15); results are presented in Table I. The T_m data show that the 10-substituted aminoalkyl derivatives (VI and VII) bind and stabilize the DNA helix toward the thermal denaturation, while the anilino derivatives (VIII–XIII) have little or no effect on the T_m of the DNA molecule.

The effect of benzonaphthyridine derivatives (VI–XIII) on *E. coli* DNA-dependent RNA polymerase *in vitro* is presented in Table II. While VI and VII are strong inhibitors, VIII–XIII exhibited a stimulation of DNA-dependent RNA polymerase. The inhibition of RNA polymerase by VI and VII appears to result from the binding of these derivatives to DNA by intercalation (indicated by T_m data), followed by an obstruction of the minor groove by the side chains, limiting the approach of the enzyme. The stimulation of RNA polymerase, on the other hand, may result from the binding of these anilino derivatives (VIII–XIII) to RNA, as has been shown for polyamines (16), which, in turn, may relieve product inhibition of the polymerase.

Compounds VI–XIII were tested against the lymphocytic leukemia P-388 test system according to the standard protocol (17) of the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health. None of the compounds screened exhibited anticancer activity patterns considered adequate to justify expanded testing (T/C = 95–110%). Activity is defined as a percent T/C of 125 or greater.

EXPERIMENTAL[1]

7-Chloro-10-[3′-(*N*-pyrrolidino)propylamine]-2-methoxybenzo[*b*][1,5]naphthyridine (VI) Trihydrochloride (General Method)—The following reaction is typical for the preparation of benzonaphthyridine derivatives (VI–XIII) listed in Table III utilizing 7,10-dichloro-2-methoxybenzo[*b*][1,5]naphthyridine (Scheme II).

To a solution of the 7,10-dichloro compound (3.35 g, 12 mmoles) in 20.0 g of phenol (previously dried at 130° for 1 hr) was added 1.84 g (14 mmoles) of III. The reaction mixture was heated to 110° for 2.5 hr with stirring and cooled. Then the reaction mixture was poured into 700 ml of dry ether saturated with hydrochloric acid gas. The yellow salt was filtered and washed well with ether and acetone. Crystallization from absolute methanol–acetone afforded 3.72 g (65%) of analytically pure VI, mp 260–261° dec., as the trihydrochloride.

RNA Polymerase Assay—Calf thymus DNA[2], *E. coli* RNA polymerase[2], the nucleoside triphosphate[2], and 8-^{14}C-ATP (50 mCi/mmole)[3] were purchased. The activity of RNA polymerase was determined by measuring the amount of ^{14}C-ATP rendered acid insoluble, as determined by the filter disk assay of Bollum (18). The incubation mixture contained, in 0.125 ml of 5 μ*M* tris(hydroxymethyl)aminomethane–hydrochloric acid buffer, pH 8.0, 1.25 μmoles of magnesium chloride, 1.25 μmoles of β-mercaptoethanol, 0.019 μmole of cytidine 5′-triphosphate (CTP), 0.019 μmole of uridine 5′-triphosphate (UTP), 0.019 μmole of guanosine 5′-triphosphate (GTP), 0.019 μmole (65,000 cpm) of 8-^{14}C-ATP, and 3.65 μg of calf thymus.

The compounds to be tested were dissolved in dimethyl sulfoxide, and 5 μl of the solution was added to the incubation mixture just prior to the

Table III—Physical Characteristics of 10-Substituted Benzo[*b*][1,5]naphthyridines

Compound	Melting Point	Yield, %	Formula	*m/e* (Relative Intensity)	Analysis, % Calc.	Found
VI	260–261° dec.	65	$C_{20}H_{23}ClN_4O \cdot 3HCl$	370 (M^+, 9), 286 (9), 84 (100)	C 50.01	50.29
					N 5.45	5.53
					N 11.66	11.74
VII	264–265° dec.	53	$C_{21}H_{25}ClN_4O \cdot 3HCl$	384 (M^+, 9) 300 (9), 84 (100)	C 51.03	51.22
					H 5.71	5.90
					N 12.04	11.92
VIII	289.5–290.5°	88	$C_{20}H_{17}ClN_4O_3 \cdot HCl$	428 (M^+, 26), 349 (100)	C 51.62	51.31
					H 3.90	3.86
					N 12.04	11.92
IX	298–300° dec.	99	$C_{20}H_{17}ClN_4O_3S \cdot HCl$	428 (M^+, 100), 349 (37)	C 51.62	51.45
					H 3.90	3.78
					N 12.04	11.86
X	286–287° dec.	75	$C_{20}H_{17}ClN_4O_3S \cdot HCl$	428 (M^+, 42), 349 (100)	C 51.62	51.26
					H 3.90	4.00
					N 12.04	11.87
XI	289–290° dec.	80	$C_{21}H_{17}ClN_4O_2 \cdot HCl$	392 (M^+, 100), 349 (25)	C 58.75	58.69
					H 4.23	4.29
					N 13.05	12.90
XII	274–275° dec.	65	$C_{21}H_{17}ClN_4O_2 \cdot HCl$	392 (M^+, 100), 377 (15)	C 58.75	58.52
					H 4.23	4.30
					N 13.05	12.85
XIII	228–229° dec.	72	$C_{21}H_{17}ClN_4O_2 \cdot HCl$	392 (M^+, 100), 377 (42), 349 (47)	C 58.75	58.62
					H 4.23	4.14
					N 13.05	12.84

[1] Melting points were obtained with a Thomas-Hoover melting-point apparatus and are uncorrected. All elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and are within ±0.4% of theoretical values, except where indicated. All mass spectra were taken on an LKB 9000 spectrometer with a source temperature of ~290°.

[2] Sigma Chemical Co.

[3] New England Nuclear.

addition of enzyme. Controls contained 5 μl of dimethyl sulfoxide. The reaction mixtures were incubated for 10 min at 37°. The acid-insoluble material from an aliquot (100 μl) of each incubation mixture was isolated by the procedure of Bollum (18), placed in 18 ml of scintillation fluid (6.0 g of 2,5-diphenyloxazole, 0.2 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene, 1400 ml of toluene, and 600 ml of methanol), and counted in a liquid scintillation spectrometer[4]. In the absence of added drug, 1.05 nmoles of 8-^{14}C-ATP was incorporated into DNA during the 10-min incubation period.

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[4] Model LS230, Beckman Instruments.

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New Method of Preparing Gelatin Microcapsules of Soluble Pharmaceuticals

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Received February 4, 1977, from the *College of Pharmacy and Allied Health Professions, St. John's University, Jamaica, NY 11439.* Accepted for publication June 13, 1977. *Present address: New Product Development, Alcon Laboratories, Fort Worth, TX 76101.

Abstract □ A new method of preparing gelatin microcapsules of soluble pharmaceuticals is described. Spherical droplets of a gelatin dispersion prepared in the drug solution were produced by the capillary method, and the droplets were congealed rapidly to yield discrete units in the form of a free-flowing powder. The microcapsules obtained were spherical in shape and showed no tendency to form agglomerates. Hardening of the microcapsules resulted in a significant reduction of the release rate without altering the reproducibility. The results indicated that the process of microencapsulation described is simple, reproducible, economical, and amenable to industrial application.

Keyphrases □ Gelatin microcapsules—method of preparation with soluble pharmaceuticals □ Microcapsules, gelatin—method of preparation with soluble pharmaceuticals □ Dosage forms—gelatin microcapsules, method of preparation with soluble pharmaceuticals

In recent years, microencapsulation has found increased use in pharmaceuticals from both clinical and therapeutic aspects.

Among the methods available for microencapsulation, the gelatin encapsulation process is the oldest and perhaps the most commonly used (1–4). Gelatin microcapsules have been prepared by complex coacervation (1, 2, 5), simple coacervation (3, 6), and emulsification (4). The emulsification process is the simplest technique, but apparently the microcapsules thus produced tend to adhere together and show poor flow properties (7). In addition, they are difficult to wet and display physical characteristics unsuitable for formulation purposes (7).

The process of encapsulation reported here makes use of the fact that aqueous dispersions of gelatin set to a gel when cooled (8).

EXPERIMENTAL

Materials—All materials were USP grade and were used as received without further purification or recrystallization. The gelatin[1] had the following specifications as provided by the manufacturer: type, B, edible; bloom, 303 g; viscosity, 58.4 mps; pH, 6.30; and moisture, 10.00%.

Microcapsule Preparation—A 30% dispersion of gelatin was prepared in a 5% aqueous solution of sodium salicylate by first soaking the gelatin in the sodium salicylate solution and then heating to about 50° to effect a homogeneous dispersion. The dispersion was maintained at 50° to prevent the gelation of gelatin, and small spherical droplets of the dispersion were prepared by a method similar to the capillary method reported previously (6).

The droplets leaving the capillary were gelled by collecting them in mineral oil USP, maintained at 5° in an ice bath. Visual observation

[1] P. Leiner and Sons, America Inc., St. Claire Shores, Mich.